OPTIMIZATION OF PHENOLIC CONTENT FROM TULSI PLANT (OCIMUM SANCTUM) BY ULTRASONIC-ASSISTED EXTRACTION: COMPARISON WITH CONVENTIONAL SOXHLET EXTRACTION METHOD

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ABSTRACT

This study is mainly about optimization of the extraction of phenolic compound from one kind of valuable herbs which is Tulsi plant (Ocimum Tenuiflorum) by comparing between ultrasonic assisted and conventional Soxhlet methods. Tulsi plants have variety of function which made it demanding in market due to the application and function of this type of herbs. Thus, this research was conducted as to investigate the total phenolic compound in ethanolic extract of the Tulsi plant leaves by verifying different parameters. The parameters that are considered for this study are the differences size particle of leaves, extraction time, temperature, and volume of ethanol solvent. The total phenolic compound in terms of concentration and mass Gallic acid equivalents was determined using HPLC. From the observation, sonication of ultrasonic assisted extraction method could improve the yield of phenolic compound and shortened the extraction times. Besides, it also can decrease the organic solvent consumption thus may lead to the increasing of pollution prevention. As a conclusion, ultrasonic extraction is one of the potential alternative methods comparing than conventional Soxhlet method.

ABSTRAK

Kajian ini adalah berkisahkan tentang proses pengekstrakan yang dijalankan dari pelbagai aspek terhadap satu jenis pokok herba yang sangat berharga iaitu Pokok Tulsi (Selasih) melalui perbandingan antara dua kaedah iatu kaedah bantuan bunyi (UAE) dan kaedah traditional (Soxhlet).Pokok Selasih ini mempunyai pelbagai fungsi dan keistimewaan untuk diguna pakai malah menjadi permintaan yang tinggi dari kebayakan pasaran disebabkan oleh keistimewaanya.Oleh itu, satu kajian telah dijalankan bagi menguji kandungan fenolik di dalam pengekstrakan daun pokok ini apabila diuji dengan faktor yang berbeza.Aspek yang dijalankan untuk kajian ini ada lah berdasarkan perbezaan saiz daun pokok selasih , masa ,suhu pengekstrakan dijalankan dan jumlah pelarut yang digunakan .Kandungan fenolik di dalam daun tersebut akan dibawa untuk dianalisa melalui alat HPLC bagi menentukan kepekatan dan berat kandungan fenolik didalamnya .Secara jelasnya, dengan kaedah bunyi, boleh meningkatkan hasil sebatian fenolik dan masa pengekstrakan lebih pendek berdasarkan pelbagai kajian .Selain itu juga, ia juga boleh mengurangkan kadar penggunaan pelarut organik seterusnya meningkatkan kadar pencegahan pencemaran alam. Kesimpulannya, pengekstrakan melalui bantuan bunyi adalah salah satu kaedah yang sangat berpotensi jika dibandingkan dengan kaedah dahulu (Soxhlet).

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LIST OF SYMBOLS

˚C	Degree Celsius
%	Percent
min	Minutes
t	Time
g	Gram
µm	Micrometer
mm	Milimeter
nm	Nanometer
mL	Mililiter
µL	Microliter
g/L	Gram/Liter
mg/mL	Miligram/Mililiter
ng/µL	Nanogram/Microliter
mL/min	Milliliter/minutes
Hz	Hertz
kHz	Kilohertz
Rt	Retention time
i.d	internal diameter
v/v	Volume/Volume
v	Volume

LIST OF ABBREVIATIONS

BHT	Butylated Hydroxytoluene
BHA	Butylated Hydroxyanisole
FYP	Final year project
GAE	Gallic acid equivalent
HPLC	High Performance Liquid Chromatography
PG	Propyl Gallate
TBHQ	tert-butylhydroquinone
TPC	Total phenolic content
UAE	Ultrasonic-assisted extraction
UV	UV-Vis Spectrophotometer

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

There is no doubt that the world today has been developing rapidly with many inventions especially in food or medical technology that facilitates our daily lives. However, almost every day, human was shocked by news of the death of many people caused by various factors especially caused by infection of food. As for example, the creation of additional materials based on synthetic materials used in many food industry cause the food looks more varied, interesting and delicious. Grigonis et al. (2005) reported that since 1949, the synthetic antioxidants is already used as to prevent the oxidations of lipids during processing of food which tendency to decrease the quality of foods besides creates deterioration of large amount of fat containing. It is supported by one research done by Juctachote & Berghofer (2004) , that the synthetic antioxidant such as Butylated Hydroxytoluene (BHT), Butylated Hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and Propyl Gallate (PG) are widely used as an antioxidant in food industry.

Unfortunately, the safety of that synthetic antioxidant has been questioned because it relating to the long terms effects beside it can be toxic (Albu et al., 2004; Boonyuen et al., 2009). Besides that, BHA was shown to be carcinogenic in animal experiments meanwhile BHT may caused internal and external haemorrhaging at high doses which contributes to death in some strain of mice and guinea pigs (Juctachote, T & Berghofer, E., 2004). Due to concern of human health, many researchers have been done with focussing more in natural resources such as herbs, for medical use especially for traditional remedies that have been used since our ancestors. In fact, antioxidant can

be divided into two types which are synthetic antioxidant and natural antioxidant. Sim, Nurestri & Norhanom (2010) said those natural antioxidants are generally alleged to be safe as they occur in plants besides more desirable than synthetic counterparts.

Moreover, during last decade, large number of plant extracts has been tested for their antioxidant activity and some promising results were obtained (Grigonis et al., 2005).Clearly, the importance of replacing synthetic antioxidant by natural ingredients from oil seeds, herbs and spices has increase due to health implications and increased functionality which improve solubility in both oil and water (Juctachote, T & Berghofer, E, 2004).There are plentiful plants have been investigated and described as potentially natural sources of antioxidant extracted from herbs , spices and fruits due to their biodegradable nature such as Rosemary (Albu et al., 2004) ,Pikul (Boonyuen et al., 2009), Okra (Khomsug et al., 2010), Tulsi leaves (Pandey & Madhuri., 2010) , Chilli & Okra (Singh et al., 2011) , Mangrove trees (Prasad et al.,2011) , oregano, thyme and many more.

Tulsi leaves are one of those wonderful herbs in this world which have variety of functions and believed could be a potential crop in medicinal use. Its Latin name is (*Ocimum tenuiflorum,* Labiates) and it also can be called as *Ocimum sanctum, Holy Basil* or *Tulasi.* Usually, it is known as " The queen of herbs " and it is most closely associated with the use in religious rituals and Ayurvedic medicine especially for Hindus (Maimes, 2004).This herbs are originally grows in India and in many Asian countries. It has been commercially in India especially for over five thousand years as a healing balm for many types of affection either body, mind even for spiritual beliefs .In simple words, the herbs are the most sacred herbs for Indian people.

Besides that, the advantages of the crops could not be denied and it is known to bestow an amazing number of health benefits. It is used to treat circulation problems beside for therapeutic uses such as lowering cholesterol and fever, removes toxins, increases stamina, improves digestion , cardiovascular - circulatory, immune and endocrine system and prevents from gastric ulcers (Maimes , 2004) . On top of that, these types of crops also can reduce tension and stress besides provides rich supply of antioxidant and other nutrients. Its herbs and oil are usually used as spice which is

widely used in cooking, additives in food, and other products for its desirable flavour and the pleasant aroma.

On top of that, Tulsi leaves also are believed that contains highly levels of natural of antioxidant such as ethanolic and phenolic content. Obviously Tulsi herb could provide fruitfully antioxidant which was more pure and natural rather than synthetic source which are widely used as additives in food, cosmetics and other products even for medicinal used. However, it is not easy to find a right method that could extract sufficiently high levels of the antioxidants in order to achieve maximum profit returns.

Among of all the process in the chemical engineering, most of it was attributed to extraction process. As we know, extraction process are lately commercially used either in large scale industrial processes or for the used in smaller scale laboratory procedures. It is nevertheless regarded now as an essential unit operation in the sophisticated armoury of modern Chemical Engineering. Several of extraction techniques such as ultrasonic assisted extraction, microwave, and supercritical fluid extraction have been developed for extraction of nutraceuticals from plants in order to shorten the time; decrease the solvent consumption besides could increase the yield and quality of extracts (Wang & Weller, 2006).

1.2 PROBLEM STATEMENT

From previous study, most of the methods chosen while extraction process occurred are usually by using conventional Soxhlet method however there is some limitations considered. Firstly, it is because of multi-step and long flow processes to be done. Moreover, the extraction process is time consuming and require large amount of solvent. As a result, there is a lot of disturbance occurred that affected the process at the same time spoil the precious bio- active compound in the Tulsi leaves.

Current studies have found alternative method to get the bio-active compound in the Tulsi leaves with some advantages which is by using Ultrasonic extraction method. The effect of ultrasound will advance the process occurred and modifying the result. Thus, not only the high extraction yields were getting but also less extraction time and low amount of solvent were used. Finally, the selection of the best method were concluded depend on the advantages and disadvantages along the process occurred.

1.3 RESEARCH OBJECTIVES

The objective of this project is:

(i) To compare the effectiveness of extraction method either using Ultrasonic assisted extraction or conventional Soxhlet method.

(ii) To evaluate the effects of different parameters in extraction process as to obtain the optimum amount of extraction yield.

1.4 SCOPE OF WORKS

During the analysis process, a few items should be scope as a guide of the project flow. Below are a few project scopes for this project as to fulfil the objectives:

(i) The extraction methods that have been used are ultrasound extraction method and conventional Soxhlet method using ethanol as solvent to get total phenolic compound in Tulsi plant.

(ii) The process have run by verifying different particle sizing at the range (315,630,800 and 1000 µm), temperature at the range of (30 - 50˚C), extraction time (20, 40, 60 and 80 min), and also the amount of solvent ratio (50-300 mL) for ultrasonic extraction method while for the Soxhlet method the parameters are verifying extraction time and size particle only.

(iii) The analysis part will evaluate by UV-Vis Spectrometer and HPLC

1.5 RATIONAL & SIGNIFICANT OF STUDY

Ultrasonic assisted extraction is one of the technique recently has been predominantly in many research. It has its own merits make it more favourable techniques in used. Based on that method, extraction time could be decrease. Besides, it only require small amount of solvent to be interact with .Thus, the production cost in terms of electricity, power and as well as utilities could be decrease at the same time we could get at high yield of product. Actually, cost of production is important issue to be considerate as for big scale applying in chemical industry.

In simple word, multi-step procedure, time consuming are remarkably to the increases the price for the final product. This is also one of the negative points taken as less attraction to the users to buy the product as the price is too high. As a conclusion, ultrasonic for extraction from plants in the application of verities aspect are a new powerful processing technology that could not only be applied safely , environmentally, friendly but also efficiently and economically. Besides that, it is more practical than the other method.

CHAPTER 2

LITERATURE REVIEW

2.1 TULSI PLANT

Tulsi plant is commonly known as Holy Basil which it is one type of plant that appears as strongly aroma, and bitter in taste. The plants normally bloom in warm regions and grow wild in the tropics climates especially in Asian countries as figure 2.1. Generally, this type of herbs is commonly can be seen in most of the botanical farm, garden even at the house corner especially for Hindus family. Pandey & Madhuri (2010) noted that Tulsi can be found in most of Indian homes and worshipped because it gives an important symbol of Hindu religious tradition. In fact, Tulsi also have precious ingredients that can be an alternative herbal treatment to treat ailments and problems such as common cold, headache, wound, bronchitis, liver diseases and many more (Pandey & Madhuri,2010). Besides that, it is widely used in varies way such as in cooking, medicine, Hindus worship and a religious tradition for thousands of years.

Beyond this wonderful herbs, there are the secret legend in varies perception by the different places, religions and history. Those plants known as the sacred herbs for Indian people and has traditionally been associated with both weddings and funerals. Besides that, Holy Basil has been revered for thousands years used in Ayurvedic systems of medicine and known as sacred plants for Indian people (Maimes, 2004) Moreover, most of them were associated Basil through their life, significance events and give meaningful symbolic pleasure for their belief. They strongly believe that Holy Basil is symbol of health, wealth and prosperity. Usually, the ladies worship this plant and pray for their husband's long life and family's prosperity.

Apart from all the religious belief, there are scientific researches proving the precious component contain in Tulsi plant. It has an active ingredient which is Phenolic content. One study led by Mukherji (1987), said that the Holy Basil oil has been found to be largely due to eugenol which is a phenolic content (1-hydroxy-2methoxy-4-allybenzene).Basically, Phenolic is one type of organic compound which have one or more hydroxyl group attached to one of the carbon atoms at the aromatic ring. Normally, this organic compound has also found as a part of tannins, which are astringent bitter plant poly phenols. In industrial applications, poly phenols have widely used as natural colorants and preservatives for foods, either in the production of paints, paper and cosmetics.

Recently, Phenolic contents have gained significant interest among various natural antioxidants extract especially in plants due to their benefit effects on health (Boonyuen et al., 2009; Khomsug et al., 2010; Singh et al., 2011). It can give benefits to our health because of the highly complex compound contain in it. In fact, phenolic content are commonly in some herbs, vegetables and fruits that can cure certain disease. There are thousands of beneficial compound which are strong antioxidant, antibacterial, antiviral, adaptogenic (agent that helps the body adapt more efficiently to stress), and immune-enhancing properties that bring positive in health. Moreover, it also can support the body's natural defence against nerves, stress and to sharpen memory (Pandey & Madhuri, 2010). Besides that, this type of herbs is used to treat our body system and enhance our circulatory problem. Tulsi plant also can supporting the heart, blood vessels, liver, and also regulates blood pressure beside blood sugar (Maimes, 2004).

Figure 2.1: Tulsi leaves

2.2 EXTRACTION

As we know, extraction process are lately commercially used either in large scale industrial processes or for the used in smaller scale laboratory procedures. It is nevertheless regarded now as an essential unit operation in the sophisticated armoury of modern Chemical Engineering. Basically, extraction is a process of separation one or more components in a mixture according to physical and chemical properties. As for example, acetic acid can be removed from the water solution by the distillation or extraction process in the presence of organic solvents, isopropyl ether (Geankoplis.C.J, 2003). Besides, herbal plant extracts are quite in high numbers of commercialize recently especially in the food, pharmaceutical and cosmetic industries (Wang & Weller .C.L, 2006). While in the pharmaceutical industry, products such as penicillin occur in fermentation mixture that are quite complex but in liquid extraction it can be done well.

There is a method that quite commercialize nowadays which is doing an ultrasonic extraction as easiest ways to get the pure compound extract naturally Additionally, there are many varieties of extraction techniques that can be done including Soxhlet extraction (Soxtec), ultrasonic assisted extraction, microwave assisted extraction, supercritical fluid extraction, accelerated solvent extraction and many more. The choice of the method would be depend on relatives cost, benefits, the effect to the environment and together the parameter that we used. Besides, a critical review was conducted to introduce and compare the conventional Soxhlet extraction and the new alternatives methods used for extraction of nutraceuticals from plants whereby all practical issues of each extraction techniques were discussed (Wang & Weller .C.L, 2006).

In other example, one study has done by Pal.R et al (2011) shows that one type of natural plants extracts contains higher level of phenol and flavonoids content which is possible to reduce the risk of chronic diseases. Thus, generation sufficiently high levels of antioxidants of phenolic compound from Tulsi leaves by extraction process for this current study are also can be done.

2.2.1 Ultrasound- Assisted Extraction

Ultrasound is a cyclic sound pressure and that term are usually used when the frequency of the sound are above 20 000 Hz. In terms of academic , ultrasound is the process of propagation of the compression (rarefracting) waves with frequencies above the range of human hearing with associated acoustic wavelength in liquid roughly 100-0.15mm (Singh ,Fernando & Hernanendez, 2006). Normally, the upper limit for human hearing is approximately 20 000 Hz and it is due to the limitation of the middle ear. In fact, the limit are varies for a different person even for some animals such as bats ,dolphins, cats, mice and dogs which can stand in range 10-100 KHz. Contrary, those species of animals can stand upper limits frequency than human hear.

On top of that, ultrasound is happen when there is the formation and implosive collapse of bubbles in a liquid irradiated which is called acoustic cavitations .In simple words, when the sound passes through the liquid, it will create negatives pressure (expansion waves) and positives pressure (compression waves).Thus the bubbles will grow and recompress the solvent and solute vapour thus make it well extracted. According to Alupului, Calinescu, & Lavric (2009), the kinetic energy affected the whole volume following the collapse cavitations bubbles near wall or interfaces thus improving the mass transfer across the solid-liquid interfaces. It has been reported by Kadkhodaee & Hemmati-Kakhki (2003) that, the sound waves were affected on the extraction process through the formation and asymmetrical collapse of micro cavities in the vicinity of the cell walls leading to the generation of micro jets rupturing the cells.

The technique is commonly used in industry as to clean the optical parts, jewellery, surgical instrument, lenses and biological cells which bacteria can be disintegrated. In industrial fields, ultrasonic are commonly used to find flaws material in a mixture.One study led by Ishtiag.F (2009) said that ultrasound also has been used in life sciences such as medical imaging, diagnostics ,and fermentation process.

Actually, since in 19th decades, ultrasonic extraction has been prepared for many kind of research unfortunately on 20th was just start to begin. Then, the study in the fields of ultrasound has been widely applied in many kinds of level involving in industrial, food department, laboratory, plant, medicine, science, and many more. Basically, there are two general designs of ultrasonic assisted extractor as figure below which are ultrasonic bath (figure 2.4 & 2.5) or closed extractors fitted with an ultrasonic horn transducer as figure 2.2 and 2.3 (Wang.L & Weller .C.L ,2006).

Figure 2.2: Ultrasonic horn transducer

Figure 2.3: Uses in Industry

Figure 2.4: Ultrasonic Bath

Source: FKKSA laboratory

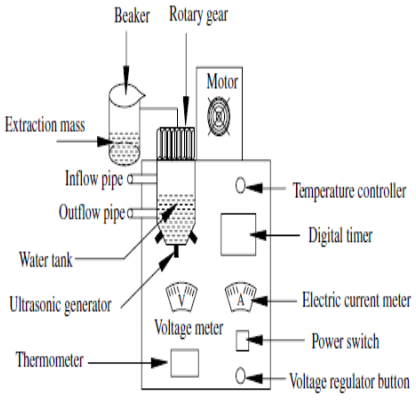

Figure 2.5: Schematic diagram UAE

Source: Ma et al., 2008, pp: 228

Additionally, there are large number of researches are now available on the extraction process as to get bio-active compound in herbs, crops ,vegetables and fruits using ultrasonic assisted extraction method (Kadkhodaee & Hemmati-Kakhki.,2003; Albu et al.,2004; Ma et al.,2008). Moreover, most of the researchers conclude that the ultrasonic assisted extraction method could reduce the extraction time in comparison with the use of conventional method.Above statement have been supported by Ishtiag et al.,2009,Wang & Weller.,2006.

2.2.2 Conventional Soxhlet extraction

Generally, Soxhlet extractor is a very useful tool for separation purposes where the analyte is concentrated from the matrix as a whole or separated from particular interfering substances. Solvent extraction of solid samples, which is commonly known as solid-liquid extraction also referred to leaching or Lixiviation in a more correct use of the physicochemical terminology, is one of the oldest process that still remains using it. Wang & Weller (2006) said that, Soxhlet is actually a standard technique which has been used for a long time and the main reference for evaluating the performance of other solid-liquid extraction method. Usually, the conventional Soxhlet extraction process is commonly applied in most of the separation process which involving oil removing from the solid matrix because of it is more straightforward and inexpensive method but also slow and tiresome (Garcia-Ayuso & Lurue de Castro, 1999).

2.2.3 Comparison Advantages and disadvantages for Ultrasonic assisted extraction and Conventional Soxhlet extraction method

Obviously, both methods have their own advantages and disadvantages according to the process requirement as shown in table 2.1.

Table 2.1: Method comparison

COMPARISON DISADVANTAGES & ADVANTAGES FOR BOTH METHODS			
NO	CONVENTIONAL SOXHLET EXTRACTION METHOD		ULTRASONIC ASSISTED EXTRACTION METHOD
1	Extraction time is long	Time	Less time as for best result
2	Large amount of solvent used	Volume solvent used	Less amount of solvent used
3	Agitation cannot be provided in the Soxhlet device to accelerate the process	Process efficient	Additional agitation or shaking is used to avoid formation solid free regions
4	Large amount of solvent requires an evaporation procedure	Evaporation process	Less temperature used avoid too much evaporation process
5	Possibility of thermal decomposition as the extraction occurs at the boiling point of the solvent for a long time	Possibility to decompose	Low tendency to decomposed due to less temperature used
6	High electricity used make high production cost	Production Cost	Lower electricity used thus make lower production cost
7	Maintaining a relative high extraction temperature with heat from the distillation flask	Temperature	Occur at lower temperature as for best results
8	No filtration requirement after leaching	Filtration procedure	Requirement of filtration process after leaching
9	Method is simple and cheap	Complexity	Additional of multiple step but still simple
10	The displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with solid matrix.	Operation efficient	-

Sources: Wang, L & Weller, C.L (2006) pg: 300-312